# A CONTRIBUTION TO THE LIFE HISTORY OF QUERCUS.

CONTRIBUTION FROM THE HULL BOTANICAL LABORATORY. XIX.

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(WITH PLATES XXVIII AND XXIX)

THE material for this study was all collected in the vicinity of the University of Chicago in the spring and summer of 1898. Gatherings of several species were incidentally made, but *Quercus velutina* Lam. (*Q. coccinea tinctoria* Gray) afforded the most complete series, and as my investigations have been devoted chiefly to this species, it is alone considered in this paper.

In dealing with material so refractory as Quercus one seldom secures a satisfactory series during a single season of collecting. The difficulties to which I here refer are due in part to the stubbornness with which the material resists the penetration of fixing agents, and in part to the remarkable uniformity with which any stage occurs in all the ovules at the same time. This bars one from an opportunity of retrieving any loss from the mismanagement of a batch of material. Detailed cytological observation has not been attempted, the object having been rather to determine the nature, order, and time of events as they occur in the gametophyte generation.

Except the brief account of Quercus by Miss Benson in her work on the Amentaceæ, I have no knowledge of any attempt to work out the life history of the genus.

The work was done under the guidance and encouragement of Dr. John M. Coulter and Dr. Charles J. Chamberlain, to whom my thanks are due.

## MATERIAL AND TECHNIQUE.

During late winter and early spring collections were made at intervals of several days, with a view to determine the stage of

development in which the several structures pass the winter, and the sequence of their development as it occurs in the spring. Beginning with May 5 and continuing to July 7, gatherings were made at intervals of two or three days, and in order that the material might be typical it was mostly taken from the upper branches of a single thrifty tree.

Owing to the protective covering of scales and glandular hairs which prevented the penetration of fixing and imbedding agents, great care was exercised in removing this so as to expose freely the structures to be investigated. Several fixing agents were tried, but chromo-acetic and picro-acetic were most satisfactory. The chromic agent preserved the material well, but rendered it more brittle and difficult to section. The picric agent is to be preferred. The chromo-acetic was a 1 per cent. aqueous solution of chromic acid with $\frac{1}{2}$ per cent. acetic acid. The picro-acetic was a saturated solution of picric acid in 70 per cent. alcohol with 0.5 per cent. acetic acid. The chromic fluid proved best when the material was wet in 95 per cent. alcohol, and quickly passed into the fixing agent. The picric was most satisfactory when used at a temperature of 80 to 90 degrees Centigrade.

Thus fixed, the material was dehydrated with successive grades of alcohol, passed into xylol, imbedded in paraffin, cut in serial sections 5 and 10 $\mu$ in thickness, and stained upon the slide. Several stains were tried. Cyanin and erythrosin proved good for early stages, Delafield's haematoxylin for archesporial stage, and fuchsin and iodine green for embryo-sac and embryo.

## THE STAMENS AND POLLEN.

Material collected in early spring (March 7) may be taken as fairly representing the stage of development in which the microsporophylls pass the winter. While the structure is well differentiated into filament and anther, the latter consists of a mass of apparently uniform cells (*figs. 1, 2*). Upon the approach of a growing temperature there occurs a rapid development and a corresponding early differentiation into the usual regions of

sporogenous, tapetal, and wall tissues, the latter comprising two to four layers of cells (*fig. 3*). These groups of cells exhibit nothing unusual either in their development or destiny. The inner wall cells are the first to break down, contributing their substance to the cells within and leaving the tapetum to form a sheath which incloses and nourishes the sporogenous cells. Before the final disintegration the cells of the tapetum show numerous prominent nucleoli, and quite generally become binucleate. The two nuclei are plano-convex or concavo-convex, with their plane or concave surfaces facing each other (*fig. 6*).

The spore mother-cells increase in size, separate, and form the usual tetrads (*fig. 4*). The mature spore has a smooth surface, is somewhat angular, and has a diameter of about 30 $\mu$. The exospore has three points of weakness, which seem to correspond to the three exterior angles of the tetrad, and which afford points of easy rupture to the tube in germination. At the time the spores are discharged, a small proportion of them show that the division into the tube and generative nuclei has occurred, the latter being the smaller and lenticular in form. Each contains a single prominent nucleolus (*fig. 5*). The subsequent history of the microspore was not satisfactorily traced. The difficulties of following it through the interval of thirteen months intervening between pollination and fertilization are multiplied by a copious development of nucleated hairs which takes place in the loculus during the interval. Three attempts were made at artificial germination of the pollen but without success. In 1898 the pollen of *Q. velutina* was shed May 17 and 18 in the vicinity of Chicago.

## THE OVULE.

The first indication of an ovule is manifest after the renewal of growth following a period of about eleven months of arrested ovarian development. *Fig. 7* represents a transverse section of the ovary on the date of March 7, and shows the condition attained during the first year of growth. The three carpels are so fused as to form three nearly distinct loculi. These communicate with one another at the base, but in the middle and

upper regions they are separated by the complete fusion of the carpels. Each loculus contains two prominent placental folds (*fig*, 7, *hh*), and numerous hairs developing from the inner surface of the carpel. *Fig*. 9 is a longitudinal section through a carpel from material collected April 21. *Fig*. 10 is a transverse section through the placental folds, which mark what may be regarded as the beginning of ovular development.

Material collected May 5 shows the first indication of the integuments (*fig*. 11, *ee*). The nucellus now rapidly elongates by active cell growth and division, especially in the basal region. The integuments push forward at a still more rapid pace, and in a short time completely inclose the nucellus. In the meantime, growth being more active in the outer angle of the base of the ovule, it is forced to an erect position.

ARCHESPORIUM.

About the time the nucellus is fairly inclosed by the integuments there is a mass of from twenty to sixty or more cells in its upper half which clearly manifests an archesporial character. This character is manifested by a larger and clearer nucleus, a coarser linin net work, and larger granules, in contrast with the nuclei of the surrounding cells, while the cytoplasm takes a much deeper stain with Delafield's heamatoxylin. In *figs*. 14–20 the extent and position of this archesporial mass is indicated. Many ovules showing this stage were sectioned and figures could be indefinitely multiplied.

In view of the usual record of events as they occur in the development of megaspore in angiosperms this archesporial mass is marked by two striking peculiarities: (1) its unusual abundance, and (2) the cells are potential megaspores as is proved by subsequent events. While multiple megaspores are known to occur in *Rosa livida*, and some of the Amentiferæ, Ranunculaceæ, Rubiceæ, and a few others, Casuarina is probably the only case hitherto known which strikingly resembles Quercus in this regard.

This archesporial stage is of relatively long duration, apparently a period devoted to the accumulation of energy to be

expended in the important changes which follow in rapid succession.

EMBRYO-SAC.

At length certain cells centrally located in this archesporial mass indicate by a thickening of the linin network, enlargement of the granules, and a coarser appearance of the nucleus, that they are entering the prophase of mitosis. The nucleoli of such cells contrast with those of neighboring cells in size and staining qualities.

Before the spirem is formed the race is usually yielded to a single vigorous cell, apparently the fourth or fifth below the surface. This cell, improving the advantage thus accorded, crowds upon its sister cells, and they in turn may soon show evidence of collapse. *Figs. 21* and *22* show a condition not uncommon, in which some of the adjacent cells are in advanced stage of deterioration, while in other instances the neighboring cells show little evidence of breaking down when the spirem is formed in the fertile megaspore (*figs. 24, 25*). It is not unusual to find the condition represented in *fig. 23*, in which the nuclei of cells in the apical region of the nucellus, previous to the spirem stage of the fertile megaspore, contain numerous small nucleoli, presumably the fragments of a single large nucleolus common to all healthy cells, and whose fragmentation may be regarded as an early evidence of the deterioration of the cell.

Inasmuch as these more marked cells usually bear a lineal relation to each other, it might suggest the usual type of tapetal cells, potential and fertile megaspores recorded for angiosperms. The events leading to this condition, and those that follow, preclude this hypothesis; and in addition it may be said that the axial position does not always obtain. Such cells may lie side by side, or they may be separated by one or more intervening cells.

As stated above, it is usual that but a single archesporial cell proceeds so far as to form the spirem. It is, however, not always the case. *Fig. 29* shows a well-defined exception. It shows two well-formed spindles with the chromosomes arranged

upon the equatorial plane. The number and form of the chromosomes are so markedly different from those of the vegetative cell that they afford conclusive evidence that the divisions are of the reduction type, and in view of all the circumstances it can scarcely be doubted that it is the first division in fertile megaspores. In *fig. 31* we have a case in which two adjacent megaspores have reached the four-celled stage. Considering the large number of archesporial cells, and the frequency with which a number of these undergo early mitotic phases, it has been a matter of surprise that the phenomenon of a two-celled stage or a four-celled stage in more than one megaspore in the same nucellus does not more frequently occur. This condition, however, we may better appreciate when we have learned more of the causes which determine the fate of a cell.

The first division observed in the apical region of any mature nucellus is shown in *figs. 26* and *27*. This is believed to be the spirem stage of the first division of the megaspore. The thread is small and I am unable to determine whether or not it conforms to the mode of splitting commonly ascribed to spore mother cells. Whatever weight, however, may be attached to synapsis as restricted to such division obtains here. Material collected June 4 and 5 afforded many cases of the fertile megaspore presenting phases illustrated in *figs. 21–27*. The transition to the four-celled stage of the embryo sac is accomplished with speed and marked uniformity of time in all the ovules. Material collected June 5 showed early phases up to and including the spirem stage of the first division of the megaspore to be quite common (*figs. 21–25*), while material collected two days later showed the four-celled stage of the sac with corresponding frequency.

Notwithstanding a large number of ovules in which the two-celled stage might be sought were sectioned, *fig. 28* probably illustrates the most satisfactory case of a two-nucleated sac. It shows two deeply stained masses which I take to be the two unorganized daughter nuclei of the first division of the megaspore, and in no case did I find two nuclei in the so-called resting stage.

In view of all the evidence, I cannot escape the conviction that the first division that occurs in the archesporium of the mature nucellus is quickly followed by a second division without the previous reorganization of the nuclei of the first division, and that this results in the four-nucleated embryo sac.

Not only does the space of two days seem sufficient for the transition from the one-celled to the four-celled stage, but in some instances development had exceeded this. It was in material collected June 7 that I found the most satisfactory illustration of the eight nuclei of the sac (*fig. 32*). Here the synergids and oosphere are in the micropylar region, the three antipodals in the base of the sac, and the polar nuclei in the act of fusing in the middle region. In this instance I find no evidence that the pollen tube has made its approach, yet in view of its elusive character it would be unsafe to affirm that it has not done so. Several cases of the fusion of the polar nuclei were observed, but the presence of the antipodals was usually a matter of doubt. This, however, is to be expected, when we consider the usual ephemeral nature of these cells, and since their nuclei possess no character which distinguishes them from the nuclei of the disintegrating cells of the nucellus, it is not an easy matter to identify them.

About the time the four-celled stage is reached, the embryo-sac begins to enlarge rapidly at the expense of the nucellar tissue, and by the time of the fusion of the polar nuclei, or soon after, the nucellus has almost entirely broken down. There is usually a fragment of the nucellus extending from the chalazal region a short distance up one side. This gives to the embryo-sac a somewhat pointed base, which is the only structure observed suggestive of the so-called *caecum*.

The synergids crowd well up into the micropyle, and display that striated beaked structure noted by observers in some other plants. *Fig. 34* in no way exaggerates the prominence of these striated tips, and, as Chamberlain observed in Salix, these tips may persist for a considerable time as a plug in the micropylar end of the sac.

The course of the pollen tube in relation to the synergids I have been unable to determine. In the most positive case of a pollen tube within the embryo-sac the course seems to have been between the synergids and the wall. Many cases were found of an open micropyle strewn with mucilaginous residue, indicating that the tube had passed, but a most careful search failed to reveal its presence within the sac. It is not unusual to find in the micropylar region of the sac numerous refractive starch granules, suggestive of the rupture of the tube and the discharge of the male cell, but the fusion with the nucleus of the oosphere was not observed.

### ENDOSPERM.

There is a very early and copious development of endosperm. I have no proof that this begins before the fusion of the male cell with the oosphere, yet its universally prompt appearance when the sac reaches maturity seems to me to indicate a doubtful time relation. This early appearance of endosperm is a source of perplexity in the investigation of male cells and antipodals. The nuclei of the endosperm lie free in the stratum of protoplasm about the wall of the embryo-sac, and the cells show no evidence of running in walls up to the time at which the embryo cuts off cells to form the dermatogen. *Fig. 36* shows the condition of the endosperm as it occurs in connection with the embryo illustrated in *fig. 39*.

### EMBRYO.

The embryo is anchored to the endosperm in the micropylar region by a short but distinct suspensor. The suspensor, at first a single cell, divides once by a vertical wall after the first few divisions have occurred in the embryo. *Fig. 37* shows the embryo of four cells while the suspensor is still undivided. At some time after this, and probably about the time the embryo cuts off cells to form the dermatogen, the vertical division occurs (*figs. 37–39*).

SUMMARY.

1. Winter buds show the stamens well formed, but with no perceptible differentiation of the cells of the anther. The carpels are also evident but relatively more rudimentary.

2. With the early growing conditions of spring there occurs the usual differentiation of the anther into sporogenous, tapetal, and wall cells. The sporogenous cells separate and form tetrads in the usual manner. The inner wall cells are the first to break down. The tapetal cells form a sheath about the spores, become binucleate, and break down shortly before the spore reaches maturity. A relatively small proportion of the spores show the division into generative and tube nuclei while still in the anther.

3. During the first year the carpels fuse so as to form three nearly distinct loculi each with two placental folds, upon each of which a single ovule develops, but the first indication of an ovule appears some time after the renewal of growth the following spring.

4. About the time the nucellus is inclosed by the integuments there is manifest in its upper half a mass of from twenty to sixty or more cells of distinct archesporial character. This character is evidenced by a larger and clearer nucleus with coarser linin network and larger granules, and by the cytoplasm of the cell taking a deeper stain than that of neighboring cells.

5. At length certain cells in the archesporial mass show a tendency to develop into megaspores. The race is usually yielded to a single cell which develops directly into the megaspore, the second division quickly following the first without the previous reorganization of the daughter nuclei of the first division.

6. Occasionally more than one cell in the same nucellus reaches the four-nucleate stage of the embryo sac.

7. The mature embryo-sac contains the usual two groups of four nuclei each. There occurs an early fusion of the polar nuclei, followed by a copious development of endosperm which shows no tendency to run in walls up to the time when the embryo is well differentiated.

8. The first division of the oospore is transverse, and the suspensor undergoes but one subsequent division, which is vertical.

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## EXPLANATION OF PLATES XXVIII–XXIX.

The figures are reduced from drawings traced by means of a Bausch and Lomb camera. Nos. 1, 9, 12, 13 were made with Reichert objective no. 7; the others with a Spencer or a Bausch and Lomb $\frac{1}{12}$ oil immersion with ocular combination magnifying 1200 and 1300 diameters.

FIG. 1. Longitudinal section through two stamens showing winter stage.

FIG. 2. Section of an anther, same date as above, showing cells of uniform character, more highly magnified.

FIG. 3. Section of anther of date April 22; shows differentiation into wall, tapetal, and sporogenous regions; the inner wall cells showing evidence of collapse.

FIG. 4. The formation of tetrads from the pollen mother cell.

FIG. 5. The mature spore with generative and tube nuclei.

FIG. 6. Two tapetal cells showing the binucleate and multinucleolate condition preceding final disintegration.

FIG. 7. Transverse section of the ovary showing loculi and placental folds (*hh*); March 7.

FIG. 8. A section of the placental fold more highly magnified; March 7.

FIG. 9. Longitudinal section of a carpel; marks the beginning of the ovule and the development of hairs from the inner surface of the carpel; April 21.

FIG. 10. Transverse section of the placental fold; April 21.

FIG. 11. Section of the ovule showing the first indication of the integuments (*ee*); May 5.

FIG. 12. Outline of the transverse section of the ovary showing the three loculi each containing two ovules; one loculus shows the hairs with which the cavity becomes almost completely filled; the ovules, nearly enclosed by the integuments, have assumed a position almost at right angles to the plane of the section.

FIG. 13. Outline of the section of the nucellus with its integuments; date same as *fig. 12*.

FIGS. 14–20. Longitudinal section of the nucellus showing the extent and position of the archesporial cells; dates ranging from May 13 to May 22; *fig. 20* is a section almost at right angles to the long axis of the nucellus.

FIG. 21. A row of five cells showing a tendency to develop into megaspores; the middle cell has taken the lead and the cells immediately above and below are breaking down.

FIGS. 22–25. Early prophases of mitosis in the first division of the megaspore.

FIGS. 26–27. Spirem stage of the first division of the megaspore.

FIG. 28. The unorganized daughter nuclei of the first division of the megaspore.

FIG. 29. Two megaspores in the same nucellus in act of first division, showing symmetrical spindles, with short and dense chromosomes arranged upon the equatorial plane, suggestive of reduction division.

FIG. 30. Four-celled stage of embryo sac; June 10.

FIG. 31. Two embryo-sacs in the same nucellus in the four-celled stage.

FIG. 32. Embryo-sac with the usual eight cells; shows the fusion of the polar nuclei in the middle portion of the sac.

FIG. 33. Embryo-sac with two synergids and oosphere in the micropylar region, and the polar nuclei in the act of fusion.

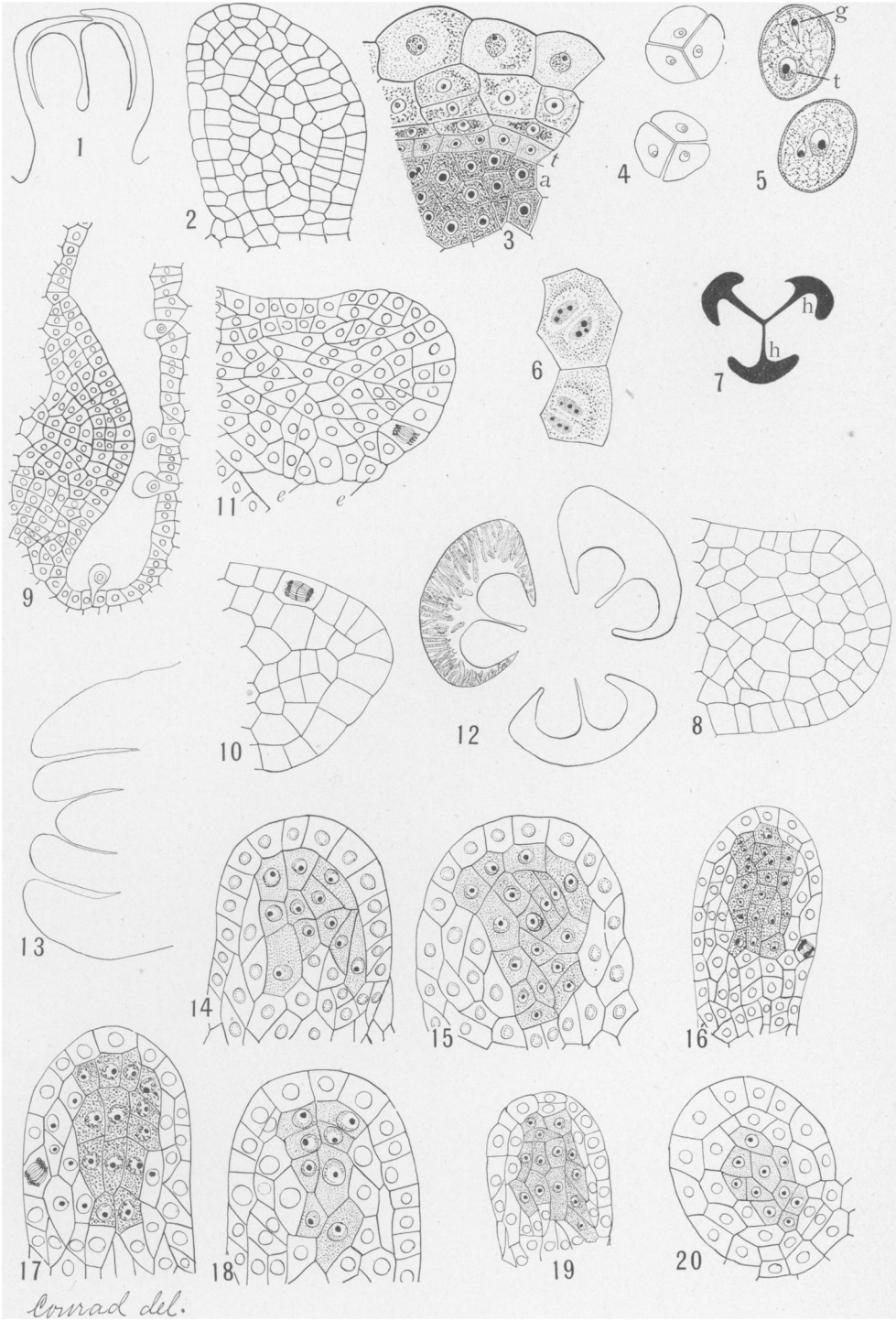
FIG. 34. Synergids with prominent striated beaks crowding into the micropyle.

FIG. 35. Synergids in the micropylar end of the sac and the oosphere suspended below.

FIG. 36. Endosperm showing the absence of any tendency to form walls at the time of embryo development represented in *figs. 38–39*.

FIG. 37. Four-celled embryo with one-celled suspensor.

FIGS. 38–39. Embryos showing periclinal division of dermatogen cells *fig. 39* more highly magnified.

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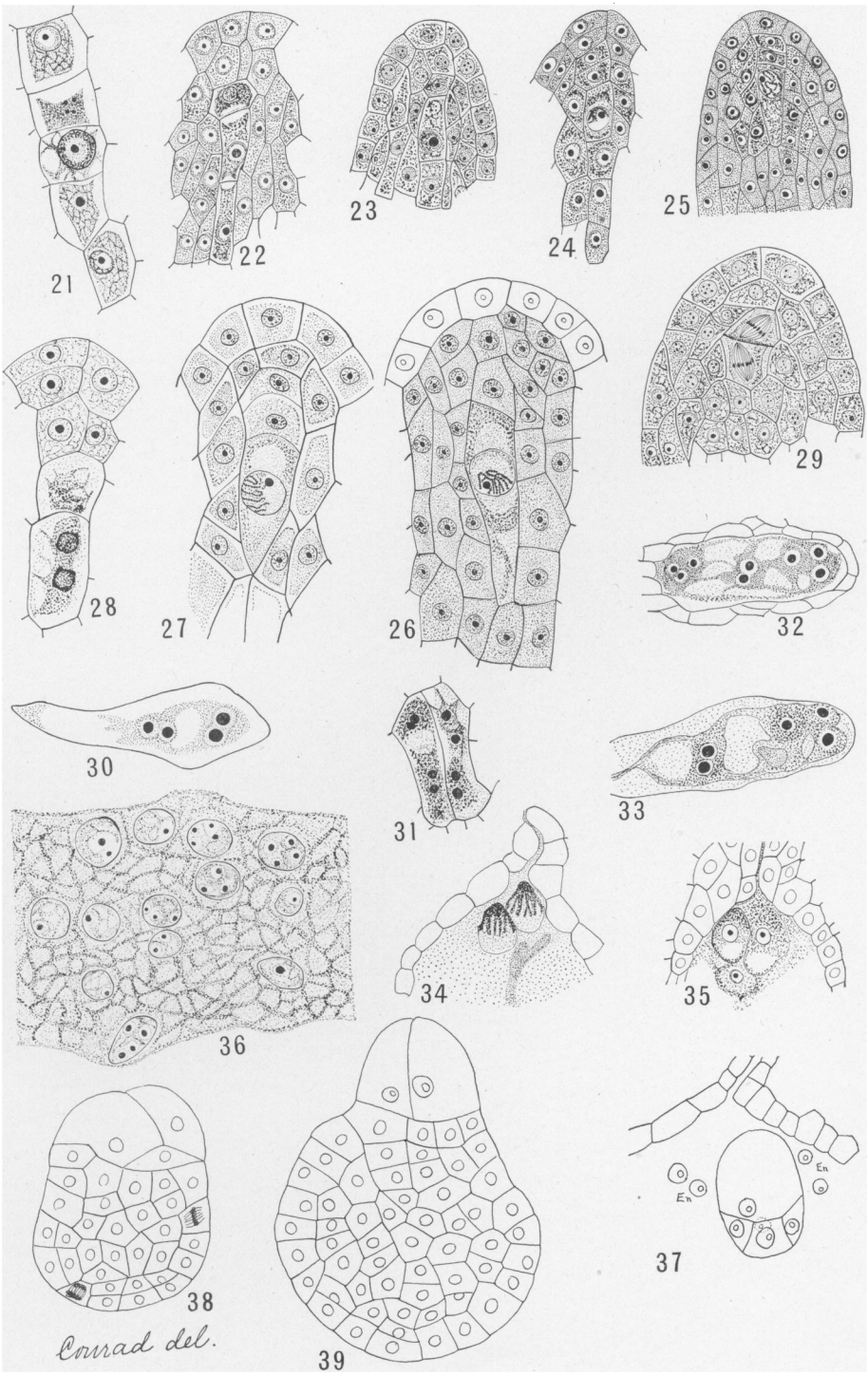


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